

## Identification of Bacterial Community and Arsenate-Reducing Bacteria Associated with a Soda Lake in Khovsgol, Mongolia

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**Abstract**—Soda lakes are extreme habitats characterized by high pH, high salt content, and occasionally associated with elevated concentrations of trace elements from volcanic origins, including arsenic (As). We characterized the bacterial community associated with a soda lake in Khovsgol, Mongolia by using both cultivation-independent and -dependent methods. Geochemical analysis of the salt evaporites present in lakeshore soils showed elevated concentrations of As, Se, and nitrate with high pH values (>8.5). Bacterial populations present in the same samples were investigated using 16S rRNA gene sequence analysis. The majority of bacterial 16S rRNA gene sequences recovered from the soda lake samples were affiliated with haloalkaliphiles in phyla Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes. Enrichment cultures exhibiting arsenate and selenate reductions were obtained. The results provide evidence that indigenous microorganisms associated with soda lake environments are capable of As and Se transformation and may contribute to the speciation and mobility of As and Se *in situ*.

**Keywords:** alkaliphile, halophile, arsenate reduction, selenate reduction

### INTRODUCTION

Saline soda lakes are naturally occurring alkaline habitats with stable high pH and high salt content. They are mostly located in semiarid to arid regions where evaporation facilitates salt accumulation in local depressions. Local geology has strong influence on the composition of dissolved salts in these lakes. Despite the extreme conditions, many saline soda lakes are highly productive and harbor diverse prokaryotic community (Sorokin and Kuenen, 2005). Microbial communities associated with soda lakes have been characterized in geographically diverse systems including: the Altai region in Russia (Foti *et al.*, 2008), the Kenyan-Tanzanian Rift Valley (Rees *et al.*, 2004), and Mono Lake, Searles Lake (California) (Oremland *et al.*, 2004, 2005) and Soap Lake (Washington) (Sorokin *et al.*, 2007) in North America.

Among the soda lake systems, Mono Lake in eastern California has attracted interests for its high abundance of arsenic from natural hydrothermal inputs coupled with evaporative concentration (Oremland and Stolz, 2003; Oremland *et al.*, 2004). Arsenic can exist in four oxidation states: -III, 0, III and V. The predominant form of inorganic arsenic in aqueous aerobic environment is arsenate, As(V), while arsenite, As(III), is more dominant in anaerobic environment. Compared to arsenate, arsenite is more toxic and mobile in the environment. Previous studies have shown that arsenic plays a central role in microbial ecology in Mono Lake. Arsenate can serve as a respiratory electron acceptor for the oxidation of organic matters, H<sub>2</sub>S or H<sub>2</sub> (Hollibaugh *et al.*, 2006; Kulp *et al.*, 2006), and arsenite can serve as an electron donor for a novel anaerobic arsenite-oxidizing photoautotrophic *Ectothiorhodospira* sp. (Kulp *et al.*, 2008) and a nitrate-reducing chemoautotrophic *Alkalilimnicola* sp. (Hoeft *et al.*, 2007). The microbial community associated with Mono Lake is capable of geochemical cycling of As, thus serving as a model system to understand microbial contributions in mobility and speciation of As in the natural environment.

Tukh Lake is an alkaline saline lake located in northern region of Khovsgol Mongolia. This lake has a unique history in that its salt evaporites, called “hujir”, were utilized for traditional medicine and served as an important source of dietary minerals for local populations. This “hujir” is commonly utilized as an ingredient in salty milk-tea which is consumed throughout the day by local nomadic herders. Previous studies analyzing the chemical composition of the hujir samples showed that they mainly consisted of macronutrients such as potassium, sodium, calcium, magnesium, chloride, and sulfate, in addition, they contained trace amounts of metals and toxic elements including arsenic (Enkhtuvshin, 2006; Barber *et al.*, 2009). In order to predict the mobilization of arsenic in aqueous environments, it is important to understand the role of microorganisms in the arsenic transformations. Here we report on the characterization of the bacterial community associated with an arsenic-containing soda lake in northern Mongolia and their potential roles in arsenic and selenium transformations.

## MATERIALS AND METHODS

### *Sample collection and chemical analyses*

Samples were collected from Tukh lake, an alkaline soda lake located in northern part of Khovsgol, Mongolia at 51°23'00.5" N, 99°28'14.9" E. Lake sediments, lakeshore soils, and salt evaporites (hujir) formed on the lake shore soil samples were collected using sterile techniques from two sites separated by approximately 30 m (site 1 and 2) in June 2008 and 2005. Samples were transported at ambient temperature and refrigerated after 10 days upon return to the laboratory. The dissolved inorganic constituents of evaporites-water extracts (1:1) were determined using inductively coupled plasma atomic emission spectrometry as described previously (Inskeep *et al.*, 2005).

Table 1. Chemical properties of Tukh Lake salt evaporites present in lakeshore soils.

Year	Sample	pH	Concentration (mg/L)* of													
			Na	B	Ca	Fe	NO <sup>3</sup> -N	K	Mg	Mn	Al	P	Ba	As	Se	
2008	Site 1	10.6	106140	6.6	48	1.1	110.7	1210	95	0.2	2.1	118.3	4.0	0.54	2.31	
	Site 2	10.6	154810	2.7	106	6.9	99.5	1059	110	0.5	4.6	61.9	2.2	0.51	2.07	
2005	Site 1	9.8	11454	59.2	15	61	0.6	642	77	1.2	47.4	1208.0	8.8	<0.05	ND**	
	Site 2	8.5	154042	3.2	122	<0.05	5.2	76	81	<0.05	2.6	7.4	0.1	2.40	ND**	

\*Concentrations and pH values were measured in 1:1 sample-water extracts.

\*\*ND: Not determined.

### *DNA isolation and 16S rRNA gene analysis*

For molecular analysis, lakeshore soil and sediment samples were collected using sterile techniques (in 2008), stored in RNAlater (Ambion, Austin, TX) immediately upon sampling and stored at  $-20^{\circ}\text{C}$  after returning to the laboratory. Total DNA was extracted from environmental samples using the PowerSoil® DNA Isolation Kit (MoBio, Carlsbad, CA). For denaturing gradient gel electrophoresis (DGGE) analysis, 16S rRNA gene fragments were PCR-amplified using *Bacteria*-specific primer 1070F and the universal primer 1392R containing a GC-clamp, and followed by separation of PCR products using DGGE. Dominant bands in the DGGE gels were purified and sequenced as described previously (Ferris *et al.*, 1996).

### *Enrichment culturing*

Enrichment culturing was conducted by inoculating sediment slurry in anaerobically prepared minimal salt AMLW medium (Hoeft *et al.*, 2007) that was modified to contain NaCl (90 g/L) and bubbled with nitrogen. The medium was amended with 10 mM lactate and arsenate, acetate and selenate, or nitrate and arsenite and incubated at  $25^{\circ}\text{C}$  in the dark. Cultures were monitored for the production of arsenite from arsenate reduction as described previously (Macur *et al.*, 2004), or the formation of red precipitate of elemental selenium from selenate.

## RESULTS AND DISCUSSIONS

The chemical analysis of salt evaporites showed elevated levels of As (0.5–2.4 mg/L), Se ( $\sim 2$  mg/L), and nitrate (0.6–110.7 mg/L) in addition to other macro salt constituents and high pH values ( $>8.5$ ) (Table 1). The chemical composition was highly variable among the evaporites examined. Concentrations of soluble As and Se as measured in 1:1 salt evaporites-water extracts were 50–240 and 50 times higher than the level of EPA drinking water standards (EPA816F-09-004), respectively. Previous study by Barber *et al.* (Barber *et al.*, 2009) examined the chemical constituents of “hujir” (salt evaporites) consumed by families near the Tukh Lake area and showed that the soluble As concentrations ranged from  $<0.05$  to 1.0 mg/L ( $n = 11$ ).

Bacterial populations associated with the soda lake sediment, lakeshore soil, and evaporites were examined by DGGE analysis of PCR-amplified 16S rRNA gene fragments (Fig. 1A). DGGE banding patterns were relatively simple, with no more than 15 distinguishable bands being observed per sample. DNA sequences of the prominent, unique DGGE bands in each sample were determined (Table 2). Phylogenetically diverse haloalkaliphiles related to Firmicutes, Proteobacteria, Actinobacteria and Bacteroidetes were identified across the soda lake samples. Many of the 16S rRNA gene sequences were closely related to cultured strains isolated from saline environments. These included an aerobic halophilic *Halomonas* sp. (Xu *et al.*, 2007), alkaliphilic heterotrophic *Nitrincola* sp. (Dimitriu *et al.*, 2005), and alkaliphilic nitrile-degrading *Nitriliruptor* sp. isolated from a

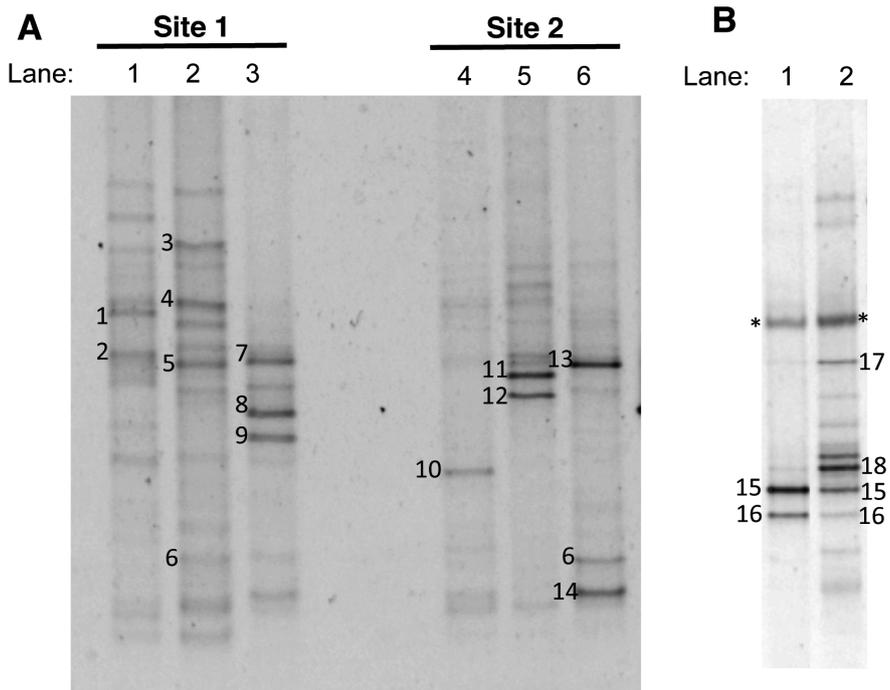


Fig. 1. DGGE analysis of 16S rRNA gene fragments from Tuxh lake samples. (A) DGGE profiles obtained from lake sediments (lane 1 and 4), lakeshore soils (lane 2 and 5), and salt evaporites (lane 3 and 6) from site 1 and 2 are shown. (B) Enrichment cultures amended with arsenate and lactate (lane 1), and with selenate and acetate (lane 2). The nucleotide sequences of the labeled bands (bands 1 to 18) were determined and are described in Table 2.

hypersaline lake (Sorokin *et al.*, 2009), a fermentative diazotroph *Bacillus* sp. isolated from soda soil (Sorokin *et al.*, 2008), and marine isolates, such as *Alkalibacterium* sp. (Ishikawa *et al.*, 2009), *Belliella* sp. (Brettar *et al.*, 2004), and *Marinimicrobium* sp. (Lim *et al.*, 2006). These cultured relatives exhibited a wide variety of physiological characteristics such as alkaliphilic, halophilic, moderately halotolerant, and strictly fermentative anaerobes to aerobic heterotrophs. All 16S rRNA gene sequences affiliated with gram-positive organisms were obtained from lakeshore soil and evaporites samples, which might indicate the presence of taxa better adapted to desiccation and oxic conditions.

Enrichment cultures were amended with arsenate or selenate to monitor the emergence of specific populations associated with As or Se transformations. After a two- to three-month incubation, arsenate- and selenate-reducing anoxic enrichment cultures were obtained which showed the production of arsenite and elemental selenium, respectively. In addition, arsenite-oxidizing anoxic enrichment culture was also obtained with nitrate as an electron acceptor.

Table 2. Sequence analysis of 16S rRNA gene DGGE bands obtained from Tukah lake samples.

Samples	DGGE band*	Closely related GenBank sequence (accession No.)	% Identity
Site 1			
Sediment	1	Uncultured $\gamma$ -Proteobacterium clone Dover340 (AY499692)	85
	2	<i>Planococcus southpolaris</i> (AJ314747)	98
Soil	3	<i>Alkalibacterium olivapovlitticus</i> (AB294175)	99
	4	<i>Bacillus alkalidiazotrophicus</i> (EU143680)	96
	5	<i>Halomonas saccharovitans</i> str. AJ275 (EF144149)	96
	6	<i>Nitiruptor alkaliphilus</i> (EF422408)	96
Evaporites	7	Uncultured <i>Pseudomonas</i> sp. clone L6B-308 (GU000235)	99
	8	Marine bacterium HEX312 (JN624847)	100
	9	<i>Rhodococcus erythropolis</i> str. cmmb1 (GU120079)	99
Site 2			
Sediment	10	<i>Belliella baltrica</i> str. BA134 (NR_025599)	99
Soil	11	<i>Nitriicola lactisaponensis</i> (AY567473)	95
	12	<i>Marinimicrobium koreense</i> (AY839869)	97
Evaporites	13	<i>Idiomarina</i> sp. C4 (EF554872)	97
	14	<i>Algoriphagus vanfongensis</i> (EF392675)	96
As(V)-reducing enrichment	15	Uncultured $\delta$ -Proteobacterium clone ML-A-19 (DQ206407)	99
	16	<i>Halanaerobium hydrogeniformans</i> (CP002304)	99
Se(VI)-reducing enrichment	17	<i>Alkaliphilus oremlandii</i> str. OhLLAs (NR_043674)	91
	18	<i>Nitriicola lactisaponensis</i> (AY567473)	98

\*DGGE band numbers correspond to those in Fig. 1.

However due to the slow growth and low biomass formation, we were unable to conduct further molecular characterization of this arsenite-oxidizing enrichment culture at that time and currently in the process of further isolation of pure cultures.

Bacterial populations in arsenate- and selenate-reducing enrichment cultures were examined by DGGE analysis of PCR-amplified 16S rRNA gene fragments (Fig. 1B and Table 2). The arsenate-reducing enrichment was dominated with two populations, whose 16S rRNA gene sequences were closely related to an anaerobic hydrogen-producing alkaliphile *Halaneorobium* sp. (Brown *et al.*, 2011) and an uncultured clone sequence from a high As-containing soda lake, Mono Lake in California (Hollibaugh *et al.*, 2006). Interestingly, the selenate-reducing enrichment also contained those two populations, suggesting the involvement of these populations in both arsenate- and selenate-reduction. Although the bands indicated by an asterisk were also shared in both enrichments (Fig. 1B), these bands are most likely a result of non-specific PCR by-products or heteroduplex formation (Ferris *et al.*, 1996) since repeated attempts to purify the bands resulted in multiple bands. A couple of additional populations were detected in the selenate-reducing enrichment, and 16S rRNA gene sequences from these bands were most closely related to those of arsenate- and organoarsenical-transforming *Alkaliphilus oremlandii* (Fisher *et al.*, 2008) and a nitrite-reducing alkaliphilic *Nitriicola* sp. (Dimitriu *et al.*, 2005) which was also detected as one of the dominant populations from the Tuxh lakeshore soil sample (Table 2 and Fig. 1A, DGGE band 11). The 16S rRNA gene sequence of the *Nitriicola*-like population in lakeshore soil (DGGE band 11) was 98% identical to that of *Nitriicola*-like population in selenate-reducing enrichment (DGGE band 18), thus they are closely related yet distinct populations. Except for these closely related *Nitriicola*-like populations, distinct populations enriched in the presence of arsenate and selenate may have been minor populations in the original sample. We are currently in the process of obtaining isolates from the enrichment cultures to further confirm their arsenate- and selenate-reducing capabilities.

This study indicated the association of diverse haloalkaliphilic bacterial with Mongolian soda lake environment, and that indigenous microorganisms are capable of As and Se transformation and may contribute to the speciation and mobility of As and Se *in situ*.

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